I3 Biodiversity

RESEARCH ARTICLE

First Record of *Gloiopotes watsoni* Kirtisinghe, 1934 parasitic on *Istiophorus platypteurs* (Shaw and Nodder 1792) from Andaman Sea with DNA barcodes

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Abstract

The knowledge of the parasitic copepods from Andaman and Nicobar archipelago of Indian EEZ is limited. The present study reports the Siphanostomoatoid parasite *Gloipotes watsoni* Kirtisinghe, 1934 from the oceanic Indo-Pacific Sailfish *Istiophorus platypteurs* (Shaw and Nodder 1792) from Indian EEZ of the Andaman Sea. 10 specimens of parasite were collected from the skin of the Indo-Pacific Sailfish during the exploratory longline survey in Andaman and Nicobar waters adds to the range extension of the parasite. Molecular marker based taxonomical annotation using Mitochondrial 18S rDNA sequencing confirmed the identity of the *G. watsoni*.

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Introduction

Parasites are ubiquitous, primarily surviving in a dynamic equilibrium with their host(s) and they are often overlooked in health assessments of Fishes. In fishes, parasites can cause mechanical damage (fusion of gill lamellae, tissue replacement), physiological damage (cell proliferation, immunomodulation, detrimental behavioral responses, altered growth) and reproductive damage (Iwanowicz, 2011). Most of the fish parasitic copepods belong to the order Siphonostomatoida Thorell, 1859 (75%) and Poecilostomatoida Thorell, 1859 (20%) (Kabata, 1988, 1992). The order Siphonostomatoida includes 57 recognized families parasitizing a wide range of hosts (WORMS, 2019). Siphonostomatoida is an order of copepods, containing around 75% of all the copepods that parasitise fishes. Their success has been linked to their possession of siphon-like mandibles and of a "frontal filament" to aid attachment to their hosts (Kabata,1992). The genus Caligus Muller, 1785 is among the most successful genera of the marine parasitic copepods, with 423 valid species (Walter & Boxshall, 2019), and they have characteristic lunules on their frontal plates (Kabata, 1988). Hewitt (1964) published an account of *Gloiopotes huttoni* and *G. watsoni* as its synonym and attributed the differences to variation within the species. Later, Cressey (1967) showed

that the two are actually distinct and should be considered separate species.

The studies of parasitic copepod fauna on marine fishes from the waters around mainland India are well documented. These studies include those of Rao (1951), Gnanamuthu (1951), Rangnekar (1961), Silas & Umerkutty (1967), Pillai (1985) and Asok-Kumar (1990). However, our knowledge of the copepod parasites of large pelagics of the Andaman and Nicobar waters is very limited to Pradeep et al. (2016, 2017 a,b). In this perspective, the present study reports a new record of *Gloiopotes watsoni* Kirtisinghe, 1934 parasitic on the Indo-Pacific Sailfish, *Istiophorus platypteurs* (Shaw and Nodder 1792) from the Andaman and Nicobar waters of the Indian EEZ along with its molecular confirmation.

Materials and Methods

Parasite specimens for the present study were collected from *Istiophorus platypteurs* caught by exploratory horizontal longlining from Andaman and Nicobar Islands waters (Figure 1) during the survey voyages of the vessel, MFV Blue Marlin of the Fishery Survey of India (FSI). Sampling was undertaken during February, 2016.

The copepod parasites were collected from the body below the dorsal fin of the Indo-Pacific Sailfish carefully by using fine forceps washed in fresh water and preserved in 70% ethanol for further analysis at the shore laboratory. Later in the laboratory, few specimens were cleaned in Lactic acid and the appendages were dissected using fine needles for detailed study. The specimens were photographed with stereo zoom microscope (LEICA M 205, DFC 500). The identity of the parasite specimens was established based on the keys to the family Caligidae provided by Kirtisinghe (1934), Cressey (1967) and Pillai (1985) and confirmed through DNA barcoding.

The genomic DNA was extracted using DNeasy Blood & Tissue kits (Qiagen. Inc) following manufacture's protocol and 18S rDNA was PCR amplified using primers 18Sf (5'- TACCTGGTTGATCCTGCCAG-3') and 1282r (5'– TCACTCCACCAACTAAGAACGGC-3') (Huys et al., 2006). Thermal cycling parameters in PRMA-96 HIMEDIA PCR included the following: an initial denaturation at 95˚C (3 min.), followed by 50 cycles of 95˚C (1 min.), 45-55˚C annealing temperature (1 min.) and 72˚C (1 min.) with a final extension at 72˚C (5 min.). The purified PCR Products were labelled using Big Dye Terminator V.3.1cycle sequencing kit (Applied Biosystems Inc.) and sequenced bi-directionally using ABI 3730 capillary sequencer using the primers 18Sfand 1282r. Partial sequences were generated and were submitted to the Genbank data base (**KY172948**). MEGA 6 software (Tamura & Nei, 1993; Tamura et al., 2013) was used for

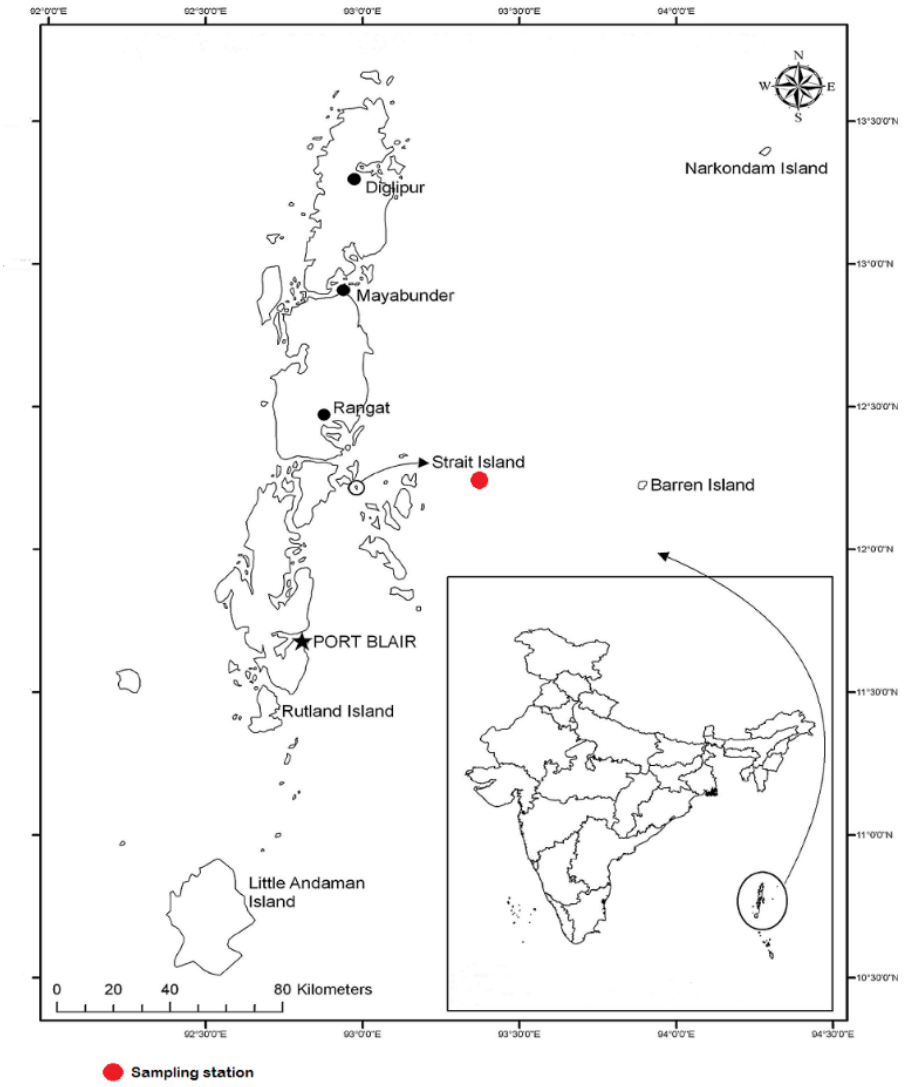

Figure 1. Map showing the Sampling station.

creating a phylogenetic tree. The voucher specimens are preserved in the museum of the zonal base of Fishery Survey of India, Port Blair.

Results

Systematics

Order Siphonostomatoida Thorell, 1859

Family Caligidae Burmeister, 1835

Genus *Gloiopotes* Steenstrup & Liitken, 1861

Gloiopotes watsoni Kirtisinghe, 1934

Gloiopotes watsoni Kirtisinghe, 1934, p. 167, figs. 1-17; Cressey, 1967, p.7, figs. 38-39; Pillai,1985, p.481, figs.162-163

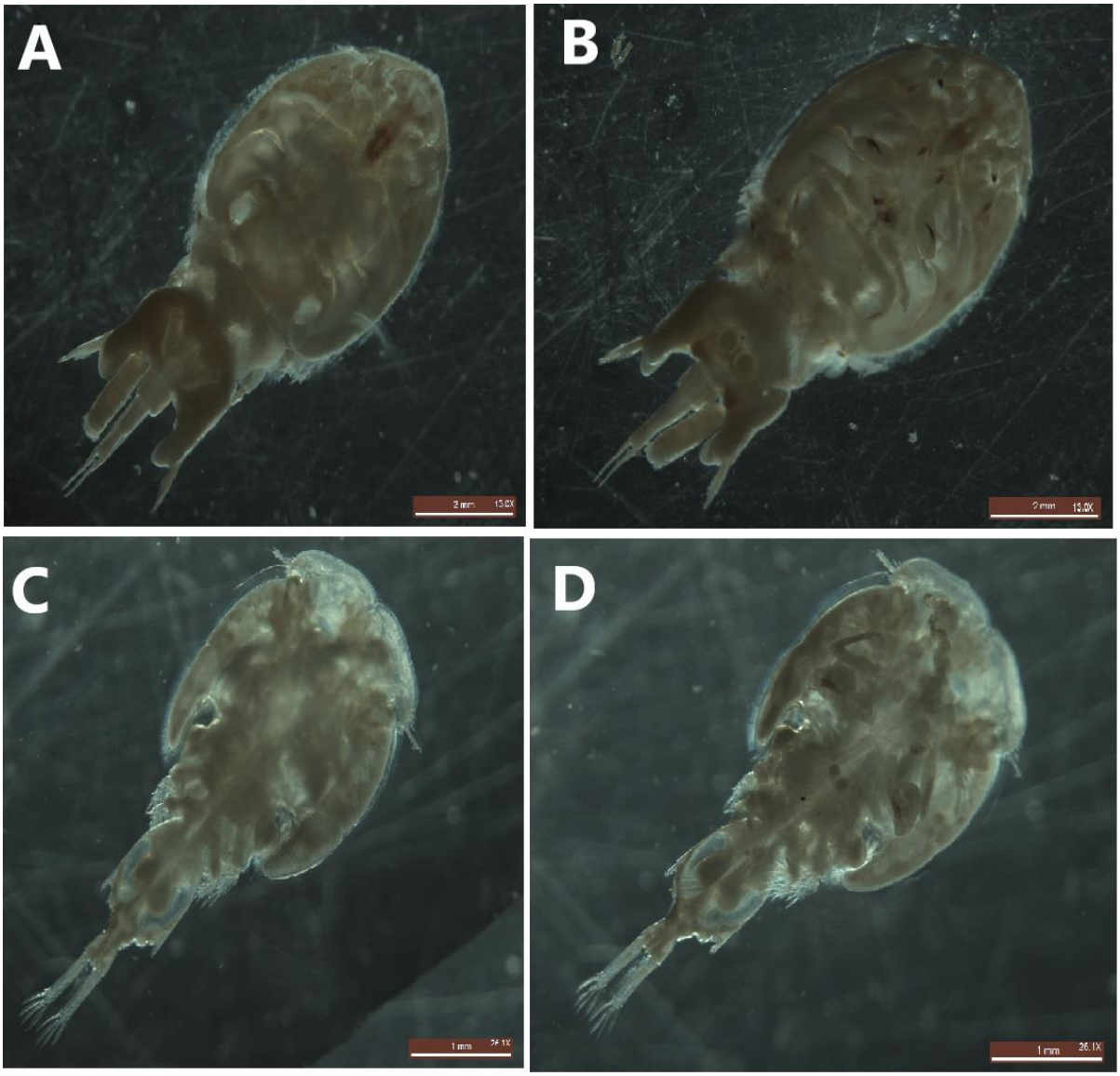

Figure 2. ***Gloiopotes watsoni*.**

A- Dorsal view (Female), **B**- Ventral view (Female), **C**-Dorsal view(Male), **B**- Ventral view(Male).

Ten specimens (6 males and 4 females) of parasites were collected from the body below the dorsal fin of a male Indo-Pacific Sailfish *Istiophorus platypteurs* (Shaw and Nodder 1792) of Fork Length (FL) 213 cm and weighing 45 Kg caught during exploratoryhorizontal longlining surveys in Andaman and Nicobar waters during February 2016 voyage. The Geographic location of the specimen collected was Lat. 12° 18' N and Long. 093° 25' E at a depth of 1748 m. The total length of the male *G. watsoni* specimens (Figure 2A) varied from 4.5 to 5.87 mm and that of females (Figure 2B) from 6.5 to 8.2 mm (excluding the egg strings). According to Cressey (1967) *G. watsoni* can easily be distinguished by observing the genital segment which is wider than long. The length of 5th leg extends up to the end of abdomen. In males the genital segment is nearly square in shape. The specimens sampled during this study had the

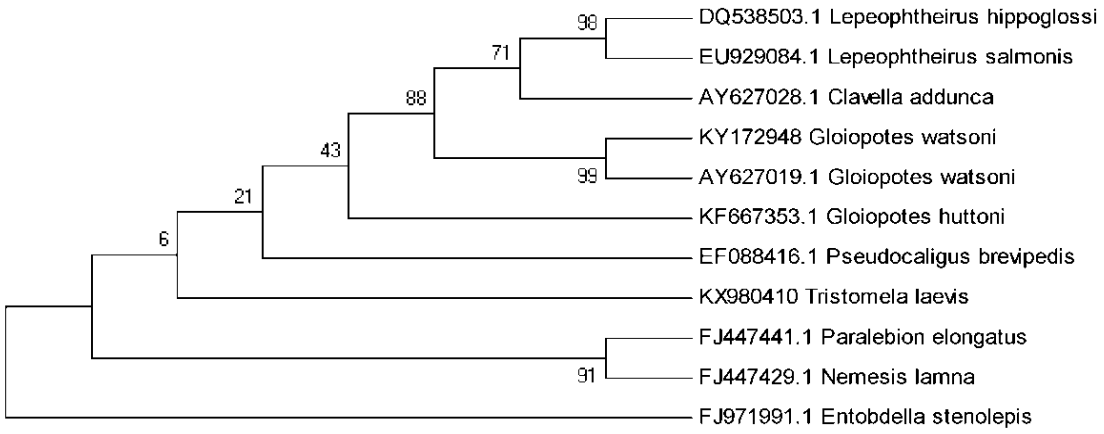

Figure 3. Neighbour Joining (NJ) phylogenetic tree of *Gloiopotes watsoni* Kirtisinghe, 1934 and related species inferred from mitochondrial 18S rDNA gene sequences (KY172948-Andaman Isolate).

above features of *G. watsoni* as described by Cressey (1967). Further, the species was confirmed using Mitochondrial 18S rDNA sequencing (Genbank accession No. **KY172948**).

Phylogenetic Analysis

Additional sequences of species of the genus *Gloiopotes* were downloaded from the NCBI database for analysis to create phylogenetic tree (Figure 3) using MEGA 6 software. Initial tree(s) for the heuristic search was obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pair-wise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The final sequence length used for further phylogenetic analyses was 407bp. The Neighbor-Joining methods resulted in phylogenetic trees (Figure 3), *G. watsoni* grouping with the *Lepeophtheirus hippoglossi*, *Lepeophtheirus salmonis* and *Clavella addunca*.

Discussion

Gloiopotes watsoni was reported from various fish hosts distributed in the Indo-Pacific area. They are known to infect 10 varieties of teleosts like *Istiophorus brevirostris, H. gladius, Xiphias zeugopteri, Makaira indica, M. nigricans, M. mazara, Tetrapturus mitzukur, T marlina, T. audax* and *Istiophorus orientalis* (Pillai, 1985). This parasite is also reported from carcharhinid sharks by Gnanamuthu (1951) from Ramaeshwarm, south India. Asok-Kumar (1990) reported the species from *Eulamia melanoptera* along Kochi, South India. The present study reports *G. watsoni* parasitic on Indo-Pacific Sailfish *Istiophorus platypteurs* (Shaw & Nodder 1792) from Andaman and Nicobar waters of the Indian EEZ as a new record.

Hewitt (1964) placed *G. watsoni* in synonymy with *Gloiopotes huttoni* (Thomson, 1890) and attributed the differences to variation within the species. Later, Cressey, 1967 concluded that the two are actually distinct and should be considered separate species. In females *G. watsoni* the genital segment is wider than long whereas it is longer than wider in *G. huttoni*. The posterior lobe is shorter in *G. watsoni* and in *G. huttoni* the posterior lobe of the genital segment extends nearly as far as the 5th leg. In males of the two species differ in the nature of the genital segment. In *G. huttoni* the segment is longer than wide, whereas in *G. watsoni* it is nearly square.

The partial sequence of mitochondrial 18S rDNA generated 407 nucleotide base pairs. Pair-wise genetic distance values were estimated based on 18S rDNA sequences using MEGA 6 software. Neighbour Joining (NJ) tree was created to provide a graphic representation of the patterning of divergences. A comparison of the DNA barcode of Andaman specimen showed 99% similarity with that of *G. watsoni* (GenBank: AY627019) which was reported to be parasitic on *Makaira nigricans* caught off Port Stephens (Australia) by Huys et al. (2006). Hence, by taking into account both taxonomical and molecular techniques the specimen was confirmed to be *Gloipotes watsoni* Kirtisinghe, 1934 confirming the range extension of the species to Andaman and Nicobar waters of the Indian EEZ.

This study provides the first report of the parasite, but there is a need for extensive study on the parasites infecting the oceanic species in the Andaman and Nicobar waters of the Indian EEZ for stock identification of the host species and its biodiversity which has not received much attention in these waters.

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